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VIRUS INFECTIONS*

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AT the present time when one speaks of an infectious disease one usually thinks, because of habit, that such a disease is caused by a microörganism. As a rule, one does not stop to analyze just what one considers a micro-organism to be, nor does one usually pause very long for thought about whether an infectious disease might be caused by an agent not classified as a microörganism.

For many centuries contagious diseases have been recognized, and infection was long an obvious fact before the cause of contagion or infection was known or understood. Then came the discovery of bacteria and protozoa; still considerable work had to be done over a period of many years with these tiny animals and plants before it was realized that they had anything to do with contagion and infection. The flowering of such an idea ushered in the microbiological era in infectious disease when it was firmly established that these maladies are caused by bacteria, fungi, spirochetes and protozoa. Indeed, it became so firmly established that these microörganisms produce infectious diseases that it was a heresy to consider them possible of causation in any other manner.

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Long before the microbiological era in infectious diseases, a method of preventing one infectious malady had been devised and its usefulness thoroughly established. I am speaking of vaccination against smallpox. When it was shown that microörganisms cause disease, investigators attempted to find a bacterium or a protozoan parasite responsible for smallpox. In fact, many different kinds of microörganisms were described as the etiological agent of this malady, but no agreement was reached regarding any of them. In 1898, the filterable virus era was vigorously initiated by the discovery that tobacco mosaic is produced by an agent capable of passing through earthenware filters, impervious to ordinary bacteria. Shortly following this, numerous agents, including those of smallpox and vaccinia, were shown to pass such filters and to be so small that it was impossible to see them by means of ordinary microscopes.

Thus, there came into existence, in addition to bacteria, protozoa, fungi and spirochetes, a group of agents now known and spoken of as viruses. As soon as this group was recognized, there immediately arose lengthy discussions regarding the nature of its members and the character of diseases produced by them. These discussions are still in progress, but fortunately much of the mystery and misunderstanding about viruses is gradually being dissipated. Perhaps some of the mystery is due to the fact that the agents producing virus diseases are not visible. For unknown reasons, it seems difficult for certain students of infectious diseases to accept the idea that some of the most powerful agents in nature do not possess a size compatible with visibility, and they still believe that the cause of an infectious disease must remain unknown, unidentifiable and mysterious as long as it is invisible. Until such an attitude has been relinquished, it will be difficult for anyone to clear away the mystery surrounding viruses and virus diseases.

There is no mystery regarding the importance of virus maladies, because they have always been, and still are, very potent factors in the economy and physical well-being of all forms of life. Indeed, from the highest form of life, man, to one of the lowest, bacteria, each stratum is involved. For instance, millions of dollars are lost each year because of virus diseases of plants and lower animals. Virus diseases of tobacco, potatoes, corn, tomatoes, beets, lettuce and sugarcane make great inroads upon our sources of income. Foot-and-mouth disease of cattle, hog cholera, swine influenza, cattle plague, fowl pox, equine encephalomye-

litis, swine pox, infectious tracheitis of chickens and avian encephalitis are also costly maladies. Man is subject to smallpox, yellow fever, measles, chickenpox, poliomyelitis and several kinds of encephalitis. Even the lowly bacterium, without which life of all forms on this world would quickly become extinct and our planet would be within a short time as barren as the moon, is subject to its own virus disease, bacteriophage. Even a casual survey of the diseases just mentioned is sufficient to impress upon anyone the reality of their importance and remove the notion that when an investigator talks about virus diseases he is dealing with something intangible and very mysterious.

The fact that there are infectious agents smaller than ordinary bacteria was discovered more or less accidentally through the use of filters specially designed to hold back bacteria while permitting the passage of their metabolic products, *e.g.*, toxins. The fact that these agents pass such filters led to their designation as filterable viruses. Filters can be made of all grades of porosity; in other words, there are filters with pores that permit the passage of bacteria, while others possess pores so small that ordinary proteins, as well as the viruses, are retained. It just happened that filters had been made to retain ordinary bacteria in order to separate their toxins from them. When such filters were used in certain kinds of investigative work, it was found that some infectious agents, capable of multiplication and for that reason not toxins, would pass through them. If other kinds of filters, that is very tight ones or ones with very small pores, had been used, the discovery of filterable viruses would have been delayed. Thus, the term *filterable virus* is somewhat misleading, because some viruses are difficult to filter, while a few very small bacteria are capable of penetrating certain of the more porous candles used in bacteriological work. At present most workers speak of viruses instead of filterable viruses, because of confusion caused by the word *filterable*.

The fact that most viruses go through filters which retain ordinary bacteria is evidence that they are smaller than such bacteria. How much smaller was not known for a long time. However, very soon it was realized that all viruses are not of the same order of magnitude, because certain of them pass filters that hold back others. Now we know the approximate size of a large number of viruses, and the diameters of these virus particles range from 250 m μ to 8 m μ . Some viruses, for instance, those of psittacosis and vaccinia, are on the border of visibility by means

of ordinary microscopes, while others, namely, those of poliomyelitis and foot-and-mouth disease, because of their small size, will never be resolved by means of ordinary light. The fact that viruses pass filters impervious to ordinary bacteria and are invisible by means of ordinary light in the unstained state, should not immediately lead one to believe that all viruses are alike in nature or that all of them are necessarily quite different from minute bacteria. Indeed, certain very minute bacteria, which are capable of cultivation on ordinary lifeless laboratory media, pass filters as readily as do the viruses of vaccinia and psittacosis. Insofar as size is concerned, there is no reason to believe that these bacteria are much more complex than are the viruses mentioned. On the other hand, when one considers the viruses of poliomyelitis and foot-and-mouth disease, it is very difficult to imagine that they are as complex as bacteria, because they possess diameters of approximately 8μ which are only slightly greater than those of certain protein molecules.

Until proper means of concentration and purification of viruses were devised, one was left with conjectures regarding the complexity of their components. Within the last ten years great strides have been made, and during that time a number of plant viruses and a few of the animal viruses have been concentrated and purified to such an extent that one is warranted in drawing certain conclusions from their chemical analyses. In addition, some of the plant viruses have been crystallized and have been shown to be large molecules of nucleoprotein containing yeast nucleic acid. When this discovery was made in 1935 by Stanley, many investigators immediately concluded that all viruses probably would finally be shown to be molecular entities, either small or large, somewhat similar to plant viruses. However, workers who have been studying the nature of vaccine virus, the infective unit of which is spoken of as an elementary body, have come to the conclusion that this particular virus is much more complex than that of tobacco mosaic, inasmuch as it is composed of several different kinds of protein, including a nucleoprotein with thymonucleic acid, neutral fats, phospholipids, and other substances as yet unidentified. In other words, some investigators are inclined to look upon the elementary bodies of vaccinia as structures possessing qualities unlike those associated with ordinary molecules.

The complexity of vaccine virus is discernible not only by chemical examination but also through immunological studies. In fact, it is at times easier to differentiate between two proteins by means of immu-

nological techniques than it is by those of chemistry. From the investigations of a number of workers, it has been definitely shown that there are several antigens associated with vaccinal infections and that they in all probability derive from the virus. Thus, it appears that there are at least two soluble antigens, one heat-stable, the other heat-labile, which occur separately or as a complex. In addition to these, there are at least two others which are intimately associated with the elementary bodies or virus, e.g., an agglutinin and a substance that gives rise to neutralizing antibodies following an infection with the virus. A similar antigenic complexity has also been found in the viruses of infectious myxomatosis and psittacosis. In the case of these two diseases, however, antigens associated with the etiological agents have not been so thoroughly investigated as they have in vaccinia. The findings mentioned are quite different from those resulting from immunological investigations of simple viruses, e.g., that of tobacco mosaic which, so far as can be ascertained, is constituted of a single antigenic substance.

Most protozoa are easily visible and some of them are cultivable on artificial media containing no living cells. This is also true of the spirochetes. Fungi and bacteria are visible, and most of them have been cultivated on lifeless media. On the other hand, viruses, in addition to being invisible, are also uncultivable on lifeless artificial media; and, for that reason, many investigators concluded that there must be something mysterious about them and that of necessity they had to be different from ordinary microorganisms. Such a conclusion does not naturally follow, because there are bacteria which have not as yet been cultivated or induced to multiply outside of a susceptible host. The bacillus which causes leprosy is an excellent example.

It is true that no virus has as yet been induced to multiply in the absence of living host cells; but such a fact should not cast a veil of mystery over the virus group, because obligate parasitism is not an unknown phenomenon even among visible infectious agents, e.g., malarial organisms which multiply in a living host and not on lifeless artificial media. If some large infectious agents are obligate parasites, there is all the more reason for minute ones to require the assistance of host cells in carrying on the functions of life and multiplication. Indeed, beyond a certain point the smaller and less complex an infectious agent, the more likely it is to be an obligate parasite; because of minute size it would not be capable of possessing all the necessary con-

stituents for autonomous existence. This matter has been discussed at length by Green who believes that all viruses are obligate parasites, even those of molecular dimensions. Obligate parasitism implies life, and Green believes that the nucleoprotein molecules of tobacco mosaic virus are living. According to him, multiplication is the only activity of which such a molecule is capable; the remaining activities necessary for such a living entity are carried out by host cells.

I admit that Green's ideas are plausible, but whether his scheme accounts for everything in the virus field is still a problem. At least there are other workers who think that some viruses, if not all, are manufactured by their host cells. According to them, there are substances in host cells which are precursors of viruses and which are converted into the virus agents through the action of proper stimuli; at the moment the only effective stimulus that is definitely recognized is some of the virus which is to be fabricated. There are excellent examples in the enzyme field to warrant such ideas; for instance, trypsinogen *in vitro* is converted into trypsin by the presence of a small amount of trypsin. At the present time Krueger and Northrop are carrying on extensive investigations with bacteriophage in attempts to show that this agent has precursors which are transformed into the virus under certain conditions. Unfortunately, as yet the results of their work are inconclusive.

From what has been said it appears that viruses are smaller than ordinary bacteria, some being much smaller, even approximating protein molecules in size, and that they do not multiply outside of a susceptible host. Furthermore, the indications are that the multiplication occurs within the involved or affected cell regardless of whether it takes place in a manner compatible with ideas regarding obligate parasitism or by fabrication through the activities of the host, aided by the processes of autocatalysis. This means that there is a very close relationship between viruses and their host cells. This close relationship between the infectious agent and the host cell undoubtedly accounts for many of the characteristic features of virus diseases. However, one must not forget that, inasmuch as there are intracellular parasites other than viruses, such features are not necessarily limited to virus diseases. On the other hand, no group of infectious agents as a whole exerts all of its forces through intracellular activity, and in that respect the virus group is unique.

As a result of the close relationship between viruses and their host cells, one of three things or a combination of these three things may occur. Rapid growth of a virus may cause immediate death of infected cells, or multiplication of the virus may first stimulate the cells and then destroy them, or, finally, the virus may act in such a manner that only stimulation of cells takes place. As one examines pathological tissues from virus diseases one sees that the things just mentioned have happened. In yellow fever, Rift Valley fever and foot-and-mouth disease the rapid growth and explosive action of the viruses lead to a picture of necrosis. In smallpox, fowl pox, vaccinia, chickenpox, and certain other virus diseases one sees early in the development of lesions only stimulation of infected cells which accounts for the formation of papules; later the stimulated cells making up the papular eruptions undergo destruction producing pustules or vesicles. Finally, in such conditions as Rous' sarcoma of chickens, Shope's papilloma, and warts, stimulation of cells is a prominent, if not the only, feature of the pathological picture.

Inasmuch as multiplication of viruses takes place within cells, it is not surprising that the phenomena just mentioned occur. In addition to this, in certain virus diseases inclusion bodies, which may or may not be made up of virus elements, are seen in the nucleus, in the cytoplasm, or in the nucleus and cytoplasm of infected cells. These inclusions are not necessarily pathognomonic of virus diseases, because some have been described in the absence of demonstrable viruses. Yet, as a rule, typical inclusions put the initiate on the lookout for a virus and at times may indicate the type of virus to be searched for; for that reason they are a great aid to virus workers.

Most physicians have been taught that inflammation is a prominent feature of infectious diseases. Inflammation occurs in virus diseases, but it is not a primary phenomenon; it is secondary to cell destruction. Many infectious diseases caused by agents other than viruses are characterized by an outpouring of polymorphonuclear leukocytes. In virus diseases, however, the inflammatory reaction is usually characterized by mononuclear cells. There are exceptions to the rule in both instances, and as yet one cannot account for the rule or the exceptions.

From what has been said about the close relationship between the host cell and virus agent, one might expect that viruses would exhibit selective localization; they do. Some viruses attack only certain hosts,

that is, they are quite host-specific. Not only are they host-specific, but frequently they attack only certain cells within these hosts. Indeed, a few investigators have attempted to classify viruses by the type of cell attacked, dividing them into epitheliotropic, neurotropic, endotheliotropic, mesotheliotropic, and pantropic agents. Such a classification, unless used very loosely, is not satisfactory, because, although there is a tendency for viruses to limit their activities to certain cells, only a few of them limit their attack to one kind of cell. Perhaps the best examples of the strict tropism of viruses are Shope's papilloma of rabbits which attacks only epidermal cells without involving the epithelial tissues of the buccal mucosa, and the wart virus of Kidd and Parsons which produces lesions in the epithelial cells of the buccal mucosa of a rabbit but not in those of the skin.

One must not entertain the idea that selective localization of infectious agents is limited to virus diseases, because a similar phenomenon is observed in other types of infection. It is well known that the meningococcus is likely to involve the coverings of the brain and cord, that the pneumococcus usually causes pneumonia, and that the typhoid bacillus generally produces an enteric infection. All the reasons for the selective localization of infectious agents, whatever their nature may be, are certainly far from being known.

Although viruses often attack more than one kind of cell, the clinical pictures produced by them for unknown reasons are usually consistent, thus enabling clinicians to make proper diagnoses with a fair amount of regularity. For instance, in spite of the fact that the viruses of measles, varicella and smallpox enter the susceptible hosts by way of the same portal and are distributed throughout the body by means of the blood, clinicians are usually able to distinguish between these three maladies. In view of the fact that viruses and other infectious agents exhibit selective localization and since some virus diseases can be diagnosed from clinical pictures alone, one must not immediately conclude that all virus diseases can be diagnosed without the aid of laboratory techniques. This is particularly true of infectious diseases of the central nervous system. In other words, one cannot forthwith differentiate between the pyogenic infections of the meninges without laboratory aid; nor can one on clinical and pathological grounds alone with any regularity correctly diagnose virus diseases of the central nervous system.

If it is difficult to differentiate one virus disease from another by

means of clinical observations alone, or if one encounters trouble distinguishing virus diseases from maladies caused by other kinds of infectious agents, how can these things be accomplished with precision? There is nothing mysterious about the matter; one goes about diagnosing virus diseases just as one proceeds in arriving at a proper diagnosis of other infectious maladies. The general principles are the same; the differences lie in the techniques used. In the first place, one attempts to isolate and identify the virus responsible for the trouble. Frequently this is possible, as in the case of psittacosis, yellow fever, rabies and influenza. At this point in the procedure the only difference between virus diseases and other infectious maladies is that one does not use ordinary lifeless media to cultivate and isolate a virus. Instead one employs living media, e.g., small laboratory animals, developing chick embryos, or modified tissue cultures.

Many of the viruses act in a characteristic way in the living media, and from this an experienced laboratory investigator obtains a clue as to the nature of the virus with which he is working. This is not unlike what the bacteriologist does when he makes cultures of microorganisms on agar plates or in broth; from the appearance of the colonies and by means of proper stains, etc., clues are gotten regarding what organism is being handled. From the appearance alone of a bacterium or from what a virus does in its living medium, one cannot be certain as to the identity of the infectious agent. In both instances it is necessary to proceed further through the use of different kinds of media, e.g., in the case of bacteria, media containing various sorts of sugar for fermentation tests, and in the case of viruses, different laboratory hosts for the establishment of the host range. Having done this, the worker has further information regarding the identity of the virus; still he may be unable definitely to classify the organism or the virus. At this point he turns for aid to classical immunological reactions. These are the same regardless of the type of infection being studied; agglutinations, precipitin reactions, complement-fixing reactions, and neutralization or protective tests are used.

Immunological principles are broad and are universally applicable provided one understands them and is ingenious enough to devise ways of applying them. Probably the first immunological phenomenon to be noted was in connection with virus diseases. Many centuries ago it was observed that individuals recovering from certain infectious mala-

dies, e.g., smallpox and measles, usually possessed a lifelong immunity. Such an enduring immunity is most striking in virus diseases, although it is known to occur in other infections. In the study of virus diseases use is made of this phenomenon in the identification of their etiological agents. Animals are infected with known viruses and after recovery they are inoculated with the unknown agent. If among the viruses used for the original inoculation there is an agent similar to the unknown, then the animal receiving that particular agent would be resistant to the unknown and in this way indicate the identity of the unknown. Such a procedure is not unique for virus studies, because it is used also for the identification of other kinds of infectious agents or their toxins.

During the last 100 years antibodies were recognized and came into use for the identification of infectious agents and for the diagnosis of infectious maladies. About forty years ago it was shown in regard to virus diseases, e.g., in the case of smallpox and vaccinia, that serum from a convalescent animal mixed with the virus responsible for the malady protects a susceptible or non-immune animal against the virus in the mixture. This procedure is known as the neutralization or protection test, and is used extensively in the identification of viruses and for the diagnosis of virus maladies. This test is not unique, because it is similar to those used for the identification of toxins, bacteria and the diseases caused by them.

The complement-fixation test was the next immunological reaction to be used in the study of viruses. Jobling, in 1906, showed that serum from an animal convalescent from a vaccinal infection upon being mixed with vaccine virus would specifically fix complement. In 1913, Paschen demonstrated that elementary bodies of vaccinia and smallpox were specifically agglutinated by serum from individuals convalescing from these infections. Then came the work of Craigie and others showing that in the case of certain virus diseases there are soluble antigens separable from the viruses themselves which precipitate in the presence of specific immune sera. These soluble antigens also fix complement under proper conditions. Thus, it is obvious that the immunological tests used in the study of virus diseases are in principle exactly like the ones employed in other infectious fields. All that is required is that one banish ideas of mystery and set oneself to the task of learning the details essential for success.

Now in regard to the treatment of virus diseases. Is there anything

peculiar about this aspect of the virus problem and is it different from those associated with other kinds of infectious diseases? With a few possible exceptions, one can immediately say, no. In spite of a few antibacterial sera and antitoxins, the treatment of bacterial infections until recently was largely expectant. Chemotherapy in the last few years has changed the whole picture. As yet, however, no great advance in this direction has been made in the virus field. It is true that certain reports have appeared stating that lymphogranuloma inguinale and trachoma are benefited by some of the sulfonamide compounds. Despite the meager results so far obtained, there is no reason to suppose that great advances in the treatment of virus diseases will not be made in the future by means of chemotherapy; indeed, this seems to be the most likely source of curative agents for this type of malady.

At the moment, the treatment of virus diseases in general is still expectant. One might inquire why serotherapy has not been successful in virus maladies. It certainly is not for the lack of intensive effort. Most of the virus diseases have been treated by immune sera, but unfortunately the results have not been encouraging. As one considers the problem in the light of what is known about viruses, one is forced to the conclusion that serotherapy of the diseases caused by these agents is not likely to yield desired results. A reason for this is found in the fact that viruses are intracellularly situated. Since antibodies do not enter cells, such a situation makes it impossible for the antibodies in therapeutic sera to reach the infectious agents. Still, one might well ask why antibodies therapeutically administered do not attack viruses as they leave infected cells on their way to attack normal cells not already involved. They do, because viruses in an extracellular location are susceptible to the action of immune sera. Then, why is it that immune sera are not efficacious in the treatment of virus diseases? A good deal of experimental evidence exists which indicates that in most virus diseases, by the time signs and symptoms of infection are manifest, all of the cells that are going to be infected in that particular host have already been entered. In view of such evidence, one would not expect serotherapy to be of great value in the handling of these maladies. Undoubtedly there may be exceptions to the statement just made, but one should demand adequate proof of the therapeutic efficacy of all anti-viral sera.

In the prevention of virus diseases there is at the present time little

to offer except quarantine measures, and several convalescent sera. Indeed, most of the quarantine measures seem fairly useless. I doubt very seriously whether measles, chickenpox, poliomyelitis, influenza and smallpox are influenced in the least by the quarantine measures now employed. Furthermore, I do not know of any that would be of use under our present social conditions. Except for making people believe that public health officials are doing something, it would seem to me that money spent on many quarantine measures might be used better in other ways. I trust there comes a time when lay people will be sufficiently informed and possessed of sufficient stamina to demand of public officials that expensive and unessential things which interfere with business and social activities be not done.

Vaccines originated in the virus field. The first successful vaccination was carried out by Jenner when he prevented smallpox by the inoculation of human beings with vaccine virus. This was accomplished on an empirical basis long before bacteria or similar agents were known to be associated with infectious diseases. Vaccination against smallpox is still the outstanding method of preventing a severe infectious malady. In spite of that, perhaps 40 per cent of the people in the United States are at this moment susceptible to smallpox. Vaccination against yellow fever has been perfected within recent years and bids fair to control outbreaks of this malady, provided public officials and the lay people are willing to see it properly used. A vaccine for equine encephalomyelitis has been developed; and perhaps in Horsfall's recent work with influenza and distemper there lies a method of preventing influenza. Tests of this influenzal vaccine are now under way, but it will be sometime yet before an answer will have been obtained. There is every reason to suppose that eventually many more virus diseases will come under control through the use of properly prepared vaccines; and it is for this reason that investigators are willing to spend many tedious hours in carrying on work that frequently yields disappointing results.

How do viruses spread in nature? There is nothing peculiar or characteristic about the spread of viruses. Most plant viruses are spread by insect vectors. A few virus diseases of man are spread in this manner, e.g., yellow fever by the mosquito. Spread of the majority of virus diseases of man that we know about at the present time, however, seems to be accomplished through contact or by means of droplet infection. Of course this means that, unless one can sterilize and keep sterile the

air which one breathes daily, it will always be difficult to control virus maladies through sanitary measures alone.

Undoubtedly some will ask why is there nothing peculiar about the epidemiology or spread of virus diseases in a population, since some of the agents causing these maladies may be fabrications of their host cells. Regardless of whether viruses are minute obligate parasites or whether they are inanimate fabrications of host cells, no virus disease has as yet been shown to arise *de novo*. Every case of a virus disease results from the entry of the virus into a host from another host. Thus, the spread of virus diseases, as is the case with other types of infectious disease, is wholly dependent upon transmission of the inciting agent from one host to another. Epidemics of virus diseases do not arise as a result of multitudinous, simultaneous foci of *de novo* fabrication of their etiological agents. A statement of this kind does not imply that new infectious agents have not come into existence during the past or that no new ones will develop in the future. Yet, so far as I know, there is no evidence to show that a single absolutely new infectious agent has come into existence during the time covered by the recorded history of man.

Upon viewing the matter dispassionately one finds that in many respects virus diseases resemble other infectious maladies. Furthermore, it is obvious that such diseases, including those displaying neoplastic phenomena, truly belong in the large category of infections. That they should be looked upon as something strikingly peculiar or even mysterious is due to a state of mind instead of factual evidence. Perhaps such a mental attitude can be accounted for to some extent by unfamiliarity with the subject.

It is true that viruses differ from other types of infectious agents; but that can be said of each of the other types. In spite of the facts that viruses are invisible, that they multiply only in living susceptible host cells, that all of them may not be alike in nature, and that some are crystalline proteins, the problems resulting from the invasion of a single host by a virus or from epidemics of virus diseases, and the general principles underlying methods of solving these problems are similar to those encountered in other infectious fields. From a practical standpoint it makes little difference at the present time whether a virus is an inanimate crystalline protein or a minute obligate parasite. In fact, the actions of bacteriologists, epidemiologists, immunologists, physicians and public health officers would not be affected immediately by final deci-

sions regarding the nature of all agents now classified as viruses. On the other hand, knowledge obtained in arriving at such decisions would be of inestimable value at some future time not only in regard to a better handling of virus diseases but also in regard to a better understanding of general biological phenomena. For that reason many virus investigators are willing to toil without thought of immediate reward in the hope of eventually making a worthwhile discovery.